

FBS06 – P30 Antigen Test for the Presence of Seminal Fluid

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1. Scope

- 1.1. This procedure is used to confirm the presence of seminal fluid on evidentiary material.

2. Background

- 2.1. P30, also known as Prostate Specific Antigen (PSA), is a glycoprotein produced in the prostate gland and is secreted in seminal fluid independently of the production of spermatozoa. The presence of p30 is used as another means of seminal fluid identification, particularly in samples with little or no spermatozoa. While p30 is not restricted to seminal fluid, its extremely high concentration in seminal fluid makes it an effective marker to confirm the presence of seminal fluid on evidence stains.
- 2.2. P30 can be detected using a chromatographic immunoassay method. A stain or sample supernatant is placed on a porous membrane in the presence of a monoclonal PSA antibody that is linked to a dye. If PSA is present in the supernatant, a PSA antigen-monoclonal PSA antibody complex will form. This complex will then migrate along the membrane where it will interact with monoclonal PSA antibody imbedded in the membrane at the test region. The antibody-antigen-antibody “sandwich” that is formed will result in a pink colored line confirming the presence of p30.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).
- 3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.

4. Materials Required

- 4.1. Seratec p30 Cards
 - 4.1.1. Note: Each lot of p30 Cards must be evaluated prior to use and periodically before expiration date. See FBQ20 for the information regarding the procedure for evaluation.
 - 4.1.2. Store cards at room temperature.
 - 4.1.3. Do not use past the expiration date.
- 4.2. TE (Tris EDTA) Buffer
- 4.3. 1.5 mL microcentrifuge tubes
- 4.4. 2.0 mL microcentrifuge tubes
- 4.5. Microcentrifuge
- 4.6. Spin baskets

5. Standards and Controls

- 5.1. The Positive and Negative Controls are cut after all the questioned stains. Control supernatants will be the last samples added to the cards in a sample set. One set of controls may be tested with each sample set. Record the results in the applicable Sample Tracking and Control Solutions (STACS) documentation.
 - 5.1.1. A portion of a known semen swab (FBR03) is cut and extracted in the proper volume and labeled as a Positive Control. This control will exhibit a solid pink line at the “T” (test) region, the “C” (control) region, and the Internal Standard 4 ng region. A positive result may be recorded at any time within the 10 minute development period.
 - 5.1.2. A portion of a sterile swab is cut and extracted in the proper volume and labeled as a Negative Control. This control will exhibit a solid pink line at the “C” (control) region and at the Internal Standard 4 ng region.

A negative result is valid if a card remains negative at the “T” (test) region for the full 10 minute development period.

- 5.1.2.1. If a sample is retained for future DNA testing, this Negative Control must accompany the sample.

6. Procedures

- 6.1. Take a cutting from a suspected semen stain or sample (see chart below for cutting sizes) and place it in a 1.5 mL or 2.0 mL microcentrifuge tube labeled with case/sample identifying information.
- 6.2. Following the chart below, add an appropriate volume of TE to samples followed by the controls.

Dimensions of Cutting (cm)	TE Volume (µL)
0.5 x 0.5	250
0.7 x 0.7	500
1.0 x 1.0	1000
¼ of swab	1000

- 6.3. Allow the samples to incubate at room temperature for at least two hours. If incubating overnight, place samples in the refrigerator at 4°C. Samples may be placed on an orbital shaker during the incubation time. If refrigerated, be sure to allow samples to come to room temperature prior to proceeding to the next step.
- 6.4. Vortex briefly and microcentrifuge for 3 minutes at maximum speed.
- 6.4.1. **Note:** If a sperm search is to be performed (FBS07 – Microscopic Examination of Spermatozoa by Christmas Tree Stain (Document Control Number: 1577)), the sample will be transferred to a filterless basket as referenced in step 6.3 of FBS07. The substrate may be retained for future testing or may be disposed of in the biohazard waste. If the cutting is retained for future DNA testing, the associated Negative Control must also be retained for future testing.
- 6.5. Unwrap the Seratec card from packaging. Take care to confirm that the lot number being used has previously been approved for casework and has not expired.
- 6.6. Label the card with the appropriate sample identification number or control name.
- 6.7. Remove the supernatant until approximately 50 µL is left in the tube, taking care not to disturb the pellet. Place the supernatant into a new appropriately labeled tube. Add 200 µL of each sample’s supernatant to the appropriate sample well of

card. Controls will be added last. Retain the pellet if proceeding to sperm search (FBS07 – Microscopic Examination of Spermatozoa by Christmas Tree Stain (Document Control Number: 1577)) or if recombining the pellet and the substrate for DNA extraction.

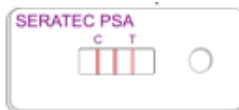
6.8. Allow the card to remain at room temperature for 10 minutes.

6.9. Read and record the results on the applicable STACS documentation. A positive result may be recorded at any time within the 10 minute period, however an inconclusive or negative result is not confirmed until the full 10 minutes has elapsed. Samples that are weak positive will be noted as such on the applicable STACS documentation. Retest inconclusive results using the remaining supernatant if possible.

6.9.1. If the pellet is recombined with the substrate for DNA extraction, the p30 Negative Control will be processed alongside the sample in addition to the required extraction reagent blank. For more information regarding the processing, analysis, and/or interpretation of this Negative Control, see the applicable SOPs.

6.10. Seratec Results:

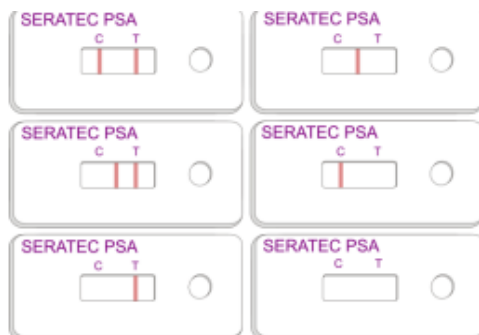
Positive:



Negative:



Inconclusive:



7. Sampling

7.1. Not applicable

8. Calculations

8.1. Not applicable

9. Uncertainty of Measurement

9.1. Not applicable

10. Limitations

- 10.1. No positive test result will be recorded after the 10 minute development period has elapsed. An unlimited detection time could lead to a false positive reaction.
- 10.2. Insufficient sample quality and/or quantity could limit the development of a positive reaction.
- 10.3. The samples must be properly diluted in order to avoid the High Dose Hook Effect. If there is an excess amount of p30 in the sample, p30 will not completely bind to the gold-labeled antibody. Free p30 will reach the test result zone and bind to the p30 antibody fixed in this zone. The binding sites of the antibody become blocked so that the p30 bound to the gold-labeled antibody can no longer bind. The formation of the sandwich complex is repressed and no pink test result line is formed, resulting in a false negative. If High Dose Hook Effect is suspected, the sample will be further diluted and retested.

11. Documentation

- 11.1. Applicable STACS documentation
- 11.2. FBU Report of Examination

12. References

- 12.1. Seratec® PSA Semiquant User Instruction Sheet
- 12.2. Quality Control of p30 Antigen Cards (FBQ20)
- 12.3. Microscopic Examination for the Presence of Spermatozoa by Christmas Tree Stain (FBS07)

12.4. FBU Quality Assurance Manual